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(54) **SYSTEMS AND METHODS FOR THE
ELECTRIC FIELD-CONTROLLED
ANESTHETIZING OF FISH**

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26, 2007.

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A22B 3/06 (2006.01)

(52) **U.S. Cl.**
CPC **A22B 3/06** (2013.01)

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USPC 119/220, 228, 219; 43/17.1, 55
See application file for complete search history.

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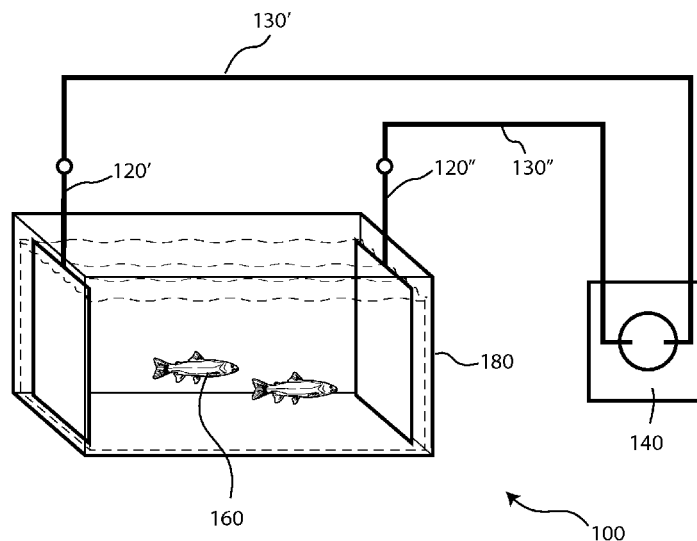
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(57) **ABSTRACT**

A system and method to induce fish anesthetizing or narcosis
is described that induces a potential field across the body of a
laboratory fish having a pair of electrodes, the waveform
generated by this potential field is approximately balanced to
reduce the introduction of anions into the solution, and a
camera is mounted to observe the activity of the fish, so that
the potential difference may be adjusted based on the state of
the fish.

5 Claims, 8 Drawing Sheets



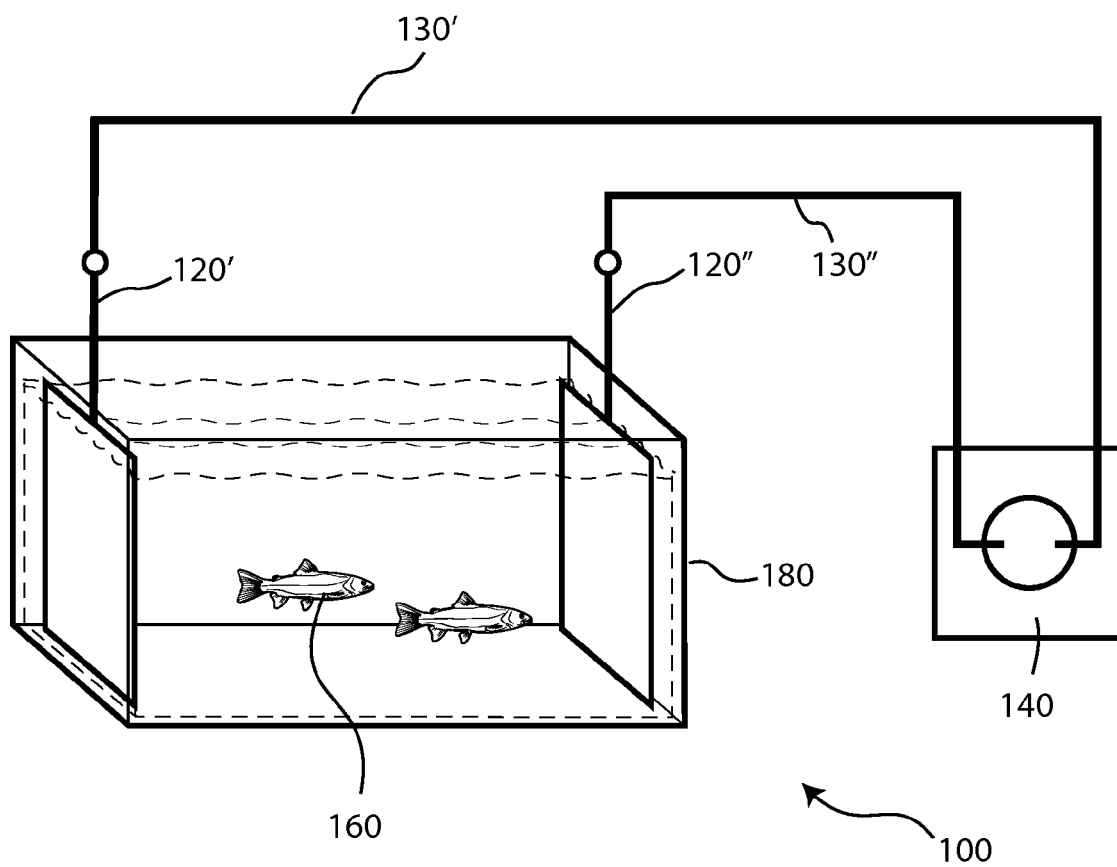
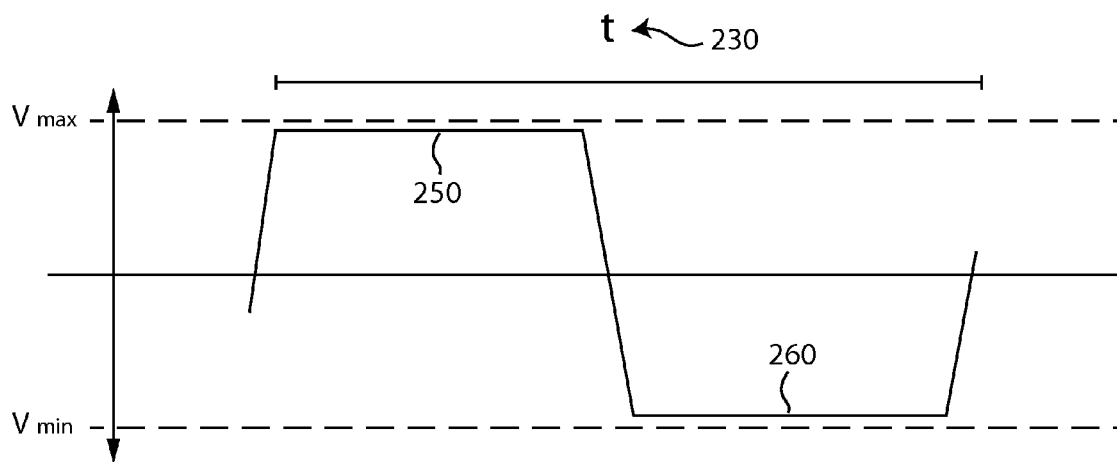
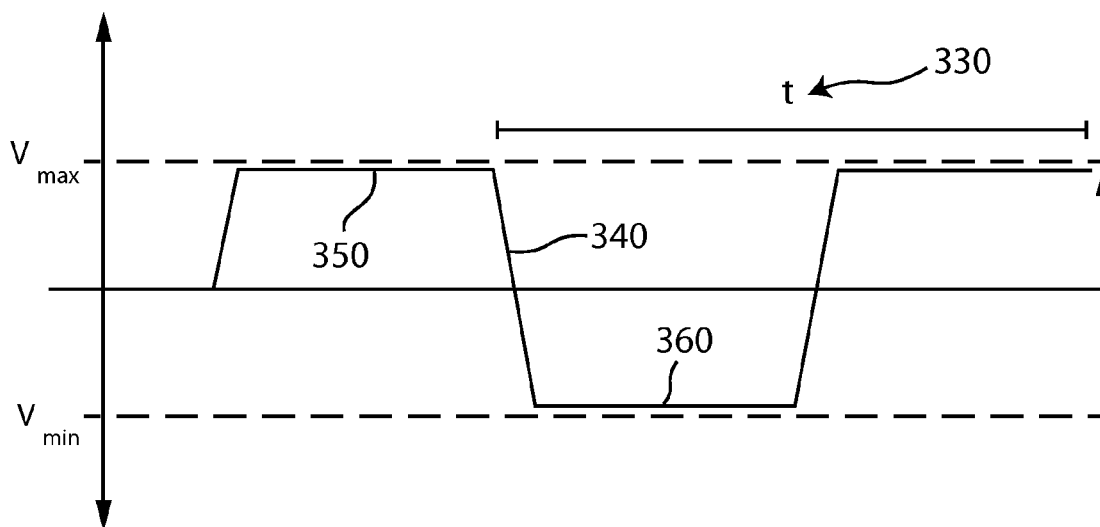
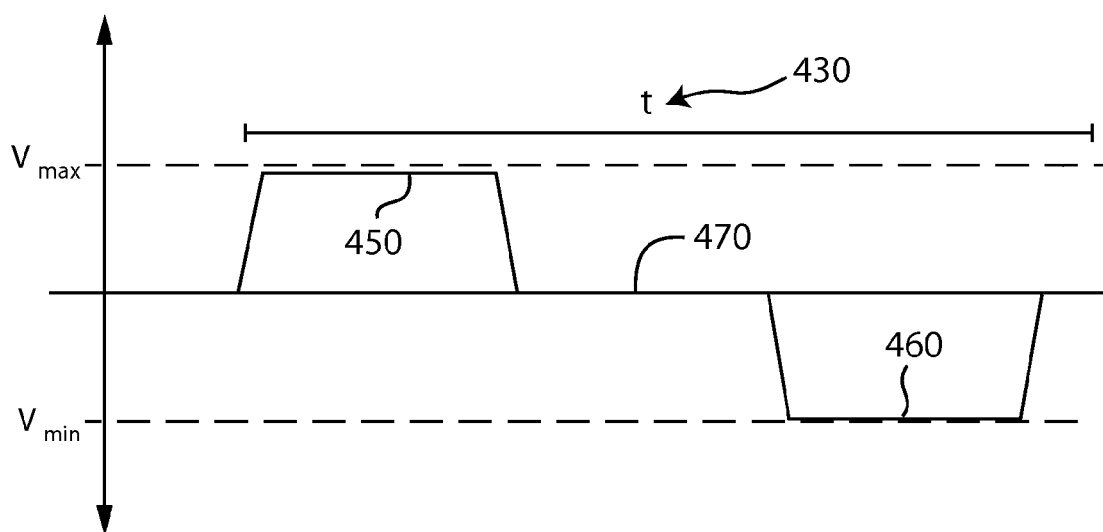


FIG.1

**FIG.2**

**FIG.3**

**FIG. 4**

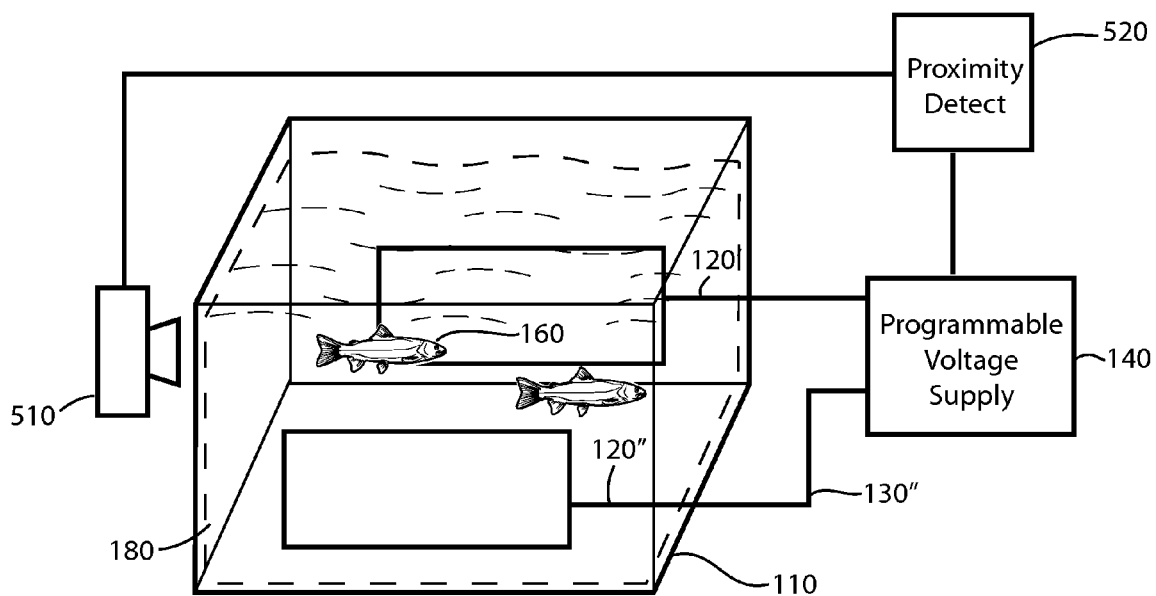
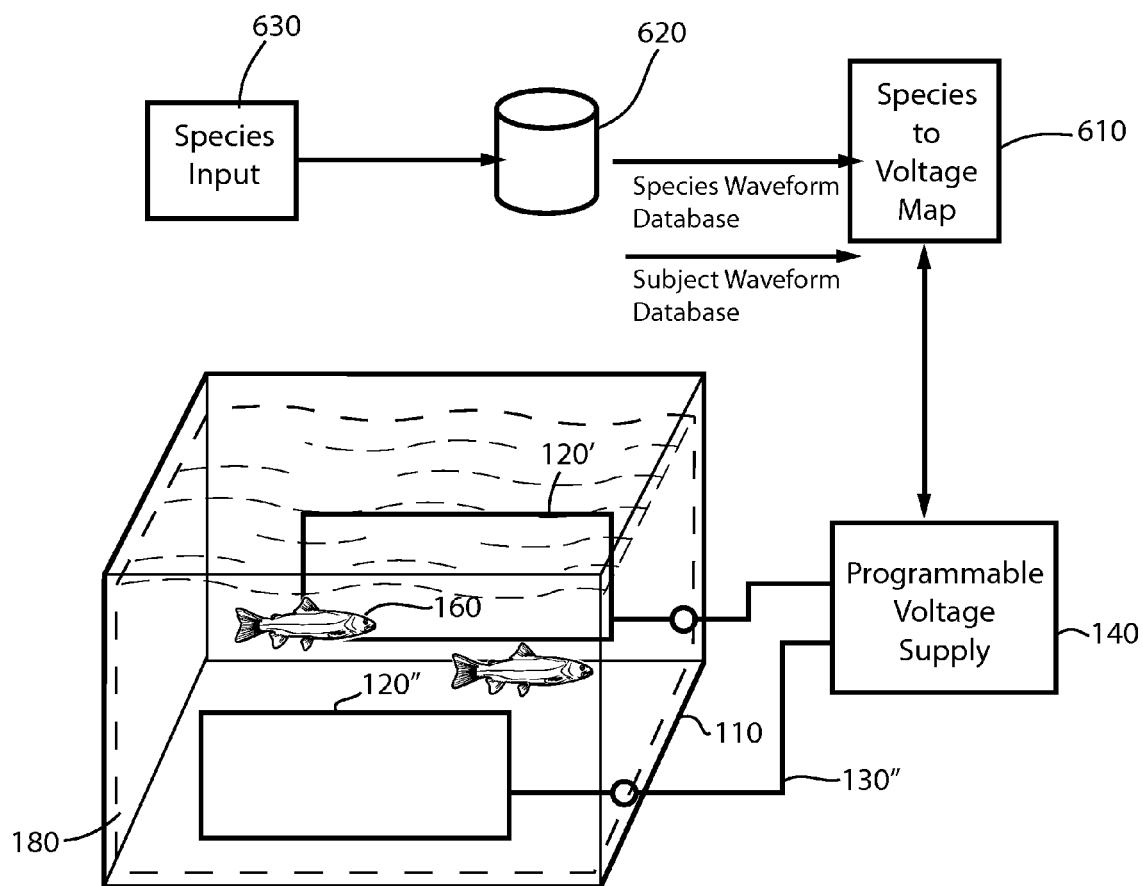
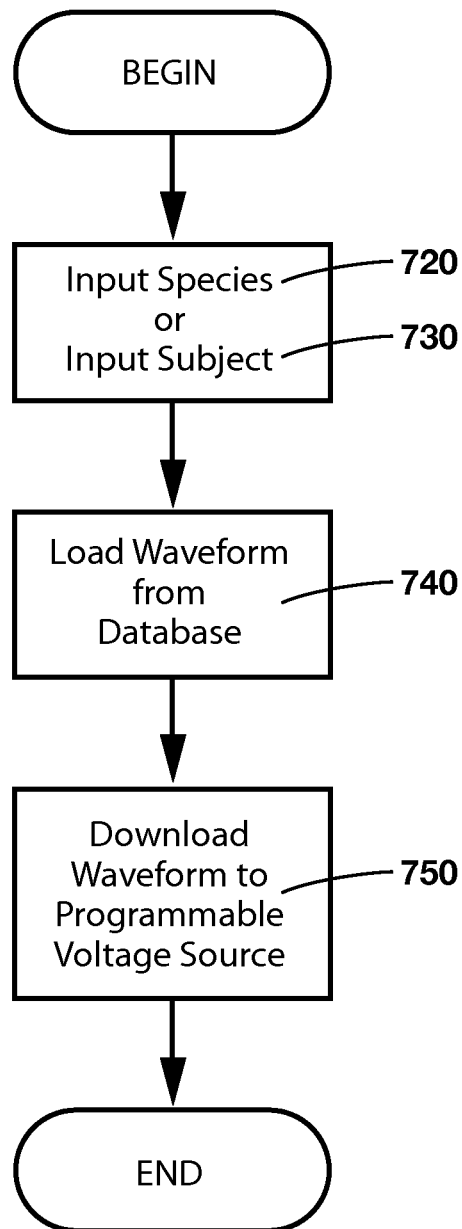


FIG.5

**FIG.6**

**FIG.7**

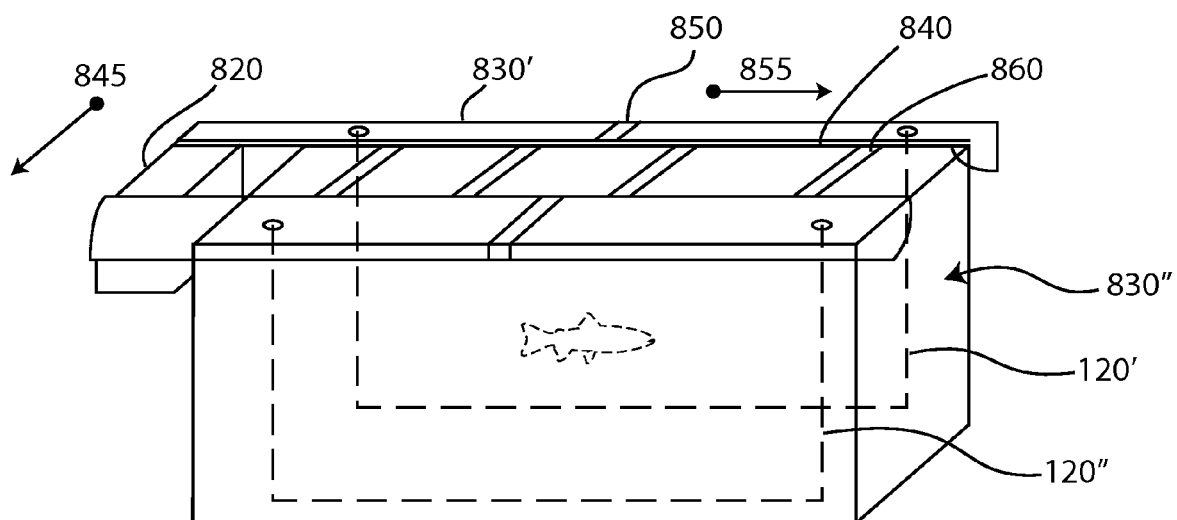


FIG. 8

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SYSTEMS AND METHODS FOR THE ELECTRIC FIELD-CONTROLLED ANESTHETIZING OF FISH

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/004,451, filed Nov. 26, 2007, the contents herein incorporated into this application by reference.

BACKGROUND

The present inventive subject matter relates to stunning and/or euthanasia of laboratory fish using electric currents and the monitoring of the laboratory fish to prevent injury thereof.

The effect of electric currents on fish are well known in the prior art and especially in the general techniques of electrofishing. Electrofishing involves the use of electric currents to attract and/or repel fish with the intent of creating aquatic barriers, sample fish barriers, and/or to increase collection yields.

It has been established that relatively small potentials that are impressed across the body of a fish invoke a flight reaction. Larger potentials result in the alignment of the fish with the electric current, or electrotaxis. Still larger potentials may result in unconsciousness or complete euthanasia of the fish. (See Introduction to Electrofishing, pages 24-26, Smith-Root, Inc. which is incorporated herein by reference).

Electrofishing has traditionally been used in freshwater lakes and streams and is the subject of U.S. Pat. Nos. 5,445,111; 5,327,854; 4,750,451; 4,672,967; 4,713,315; 5,111,379; 5,233,782; 5,270,912; 5,305,711; 5,311,694; 5,327,668; 5,341,764; 5,551,377; and 6,978,734 which are incorporated herein by reference. Also, electrofishing has been the used to stimulate yields of fishing in conjunction with the use of trawl nets as described in U.S. Pat. Nos. 3,110,978 and 4,417,301 which are also incorporated herein by reference.

Systems for controlling electricity in aquatic environments have been described in U.S. Pat. No. 5,460,123 which is incorporated herein by reference.

There are also electrofishing systems that attempt to reduce the leaching of metal ions into water due to the electrolytic action of passing current through the solution. Also systems for monitoring small laboratory fish using cameras are described in U.S. Patent Publication 2006/0018833 which is incorporated herein by reference.

Therefore in the prior art, a considerable body of work is associated with the electrification of large bodies of water to impress electric fields across larger fish, such as salmon or trout, as compared to smaller fish, that would be typically be used for the purpose of laboratory experimentation.

The use of small fish, in particular zebrafish, are popular as biological models for scientific studies. The studies involve placing fish in laboratory desktop tanks, then subjecting the fish to physical, chemical, or biological stress. This is followed by an examination of the subject fish to determine the various effects on the fish as a result of the stresses. For the purpose of this application the term "fish" will refer to any suitable aquatic laboratory fish, including, but not limited to zebrafish.

A recurring problem with the examination of laboratory fish is that they tend to be very active. Also laboratory fish are dimensionally very small. The small size of the fish combined with their activity can impair the researcher from making precise scientific measurements unless the fish is caught and inspected.

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Therefore there is a need for the laboratory researcher to euthanize laboratory fish in a relatively quick and painless manner. Ideally the state of euthanasia can be controlled for a period of time in which the measurement of the fish is needed.

To induce euthanasia an electric field is applied to the water in the tank. This, in turn, induces an electric field across the body of the fish. This amount of electricity that is passed through the fish varies based on the orientation of the fish and the magnitude of the electric field.

Low electric fields evoke a deterrent response in the fish causing the fish to be repelled from the electric field. Greater electric fields may evoke an involuntary response that results in the fish being attracted and swimming towards the anode and away from the cathode. Still larger electric fields may result in a state of narcosis being induced in the fish. Higher electric fields may result in euthanasia (i.e. death) of the fish.

Prior art solutions and techniques that induce euthanasia in fish involve the addition of chemicals to the tank. Chemicals used for euthanasia are expensive to acquire, pose a storage and maintenance problem, and are at risk of degradation. These chemicals, which are toxic to fish, may have the unfortunate consequence of skewing laboratory results during any post-euthanasia pathology. Furthermore, the researcher runs the risk of exposure to the chemicals that are used to euthanize the fish. Also, the use of chemicals to euthanize fish may be unnecessarily complex and expensive, which would result in the added cost of training specialized personnel.

Furthermore, there is also a view that the chemical euthanasia of fish may induce unnecessary pain. In response, the American Veterinary Medical Association (AVMA) Panel on Euthanasia has listed three acceptable methods of euthanasia for fish, and two conditionally acceptable methods. These methods include the use as acceptable of such chemicals as tricaine methanesulfonate (MS-222), benzocaine (related to MS-222 but less soluble in water) and barbituates.

In view of the high cost and risk of certain chemical agents that induce euthanasia in fish, there is a need to have a unit that will induce unconsciousness and euthanasia in laboratory fish without chemicals. There is also a need for a portable unit that can euthanize fish without the use of chemical agents. There is also a need for a unit that will shield the researcher from contact with potentially hazardous chemical agents. There is also a need for unit that can euthanize fish using electrical currents. There is also a need for a unit that can euthanize fish using rechargeable batteries.

There is also a need to have a monitoring system to insure that a laboratory fish does not come into contact with an anode or a cathode to reduce the risk of injurious shock to the fish.

There is also a need to reduce the leaching of anions into the solution (e.g. fish water) during fish euthanasia. In certain cases, a laboratory fish may be euthanized first by transferring to a euthanasia tank, then followed by actual euthanasia, then disposal. During operation, anions will leach from the electrodes via electrolytic action resulting in the further contamination of the water. It is therefore desirable to have a unit that minimizes the leaching of anions from an electrode during euthanasia.

Therefore, what is desired is an apparatus to immobilize fish and place the fish in the state of euthanasia. It is also desired that this apparatus operate at relatively low voltage levels. It is also desired is an apparatus that is portable. It is also desired that the apparatus prevent the leaching of anions from electrodes into the water electrodes during operation.

SUMMARY

The present inventive subject matter overcomes problems in the prior art by providing a fish anesthetizing apparatus that

induces a potential field across the body of a laboratory fish with a programmable power supply, the programmable power supply having a first output terminal and a second output terminal, a pair of electrodes, the first electrode connected to the first output terminal and the second electrode connected to the second output terminal; whereby when the pair of electrodes are inserted in the enclosed body of water, a potential field is created by the programmable power supply so that two electrodes induce an altered state in a laboratory fish. The fish anesthetizing system also includes a programmable power supply where the potential difference between the first electrode and the second electrode is an impulse signal. The fish anesthetizing system also has a programmable power supply where the potential difference between the first electrode and the second electrode creates a field that induces electronarcosis in a laboratory fish. There is also a need for a fish euthanasia system where programmable power supply creates a potential difference between the first electrode and the second electrode thereby creates a field that induces electroaxis in a laboratory fish. The fish anesthetizing system apparatus also induces an electric field across a fish with a programmable power supply, an anode and a cathode, where the anode and cathode is capable of immersion water and; so that when the anode and cathode are immersed in water, a potential difference is generated across a laboratory fish, such that the fish experiences a physiological change. The fish anesthetizing apparatus wherein the potential difference is a periodic waveform for fish anesthetizing. The fish anesthetizing apparatus also having a cyclic waveform that is a square wave. The fish anesthetizing apparatus also having the amount of the power generated in the positive and negative phases that are approximately equal. The fish anesthetizing apparatus having a waveform that is an impulse wave. The fish anesthetizing apparatus also having a maximum potential difference ranges from 0.2 V/cm to 10.0 V/cm such that electronarcosis is induced in a fish. The fish anesthetizing apparatus where the maximum potential difference ranges from 0.1 V/cm to 10 V/cm such that electroaxis or euthanasia is induced in a fish.

Also described is a method for inducing an electrical potential across a laboratory fish, the method consisting of obtaining a programmable power supply, connecting an anode and a cathode to the programmable power supply, inserting the anode and the cathode into a fish tank, increasing the potential difference between the anode and the cathode so that there is a potential difference of up to 10 V/cm across the body of the fish, decreasing the potential difference between the anode and the cathode to approximately 0 V/cm across the body of the fish; so that when the potential difference first increases and then decreases across the body of the fish, the fish experiences a physiological change. Furthermore, the method also includes inducing a potential difference between the anode and cathode such that an electric field is increased and decreased across the fish in a cyclic manner.

Also described is a system for impressing a voltage across a laboratory fish, the system which comprises a means for generating a voltage, a means for introducing the voltage into a laboratory tank, so that when the voltage is generated a potential difference is created across the laboratory fish that ranges from 0.0 to 10.0 V/cm.

Also described is a portable fish anesthetizing system having a tank cover, an electrode pair, the electrode pair configurable attached to the tank cover; a control system for creating a potential difference, the control system electrically connected to the electrode pair, so that when the tank cover is placed on a laboratory tank and the control system is activated, current flows from the control system through the

electrodes. Also shown is a portable fish anesthetizing system with a tank cover further incorporating a top part and a bottom part, the bottom part being configured to fit on the top rim of the laboratory tank and the bottom part is adjustable in the either the lengthwise or widthwise dimension so that it may conform to the top of the laboratory tank.

The portable fish anesthetizing system also has a tank cover further control system further comprises a camera, where the camera is connected to the control system, a series of fish images are recorded from fish in the tank, the control system then identifies a class of fish, and in response to the class of fish detected, adjusts the current flow; so that the fish experiences electronarcosis, electroaxis, and/or euthanasia. The portable fish anesthetizing camera system also is integrated with the tank cover.

These and other embodiments are described in more detail in the following detailed descriptions and the figures.

The foregoing is not intended to be an exhaustive list of embodiments and features of the present inventive subject matter. Persons skilled in the art are capable of appreciating other embodiments and features from the following detailed description in conjunction with the drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a system diagram of the laboratory fish anesthetizing system.

FIG. 2 is a waveform diagram of a single pulse with a continuous positive and negative opposite polarity lobes.

FIG. 3 is a waveform diagram of plural positive and negative lobes.

FIG. 4 is a modified waveform diagram with a zero-field dwell portion interposed between the positive and negative field lobes.

FIG. 5 is a system diagram of the laboratory fish anesthetizing system incorporating a proximity detector.

FIG. 6 is a system diagram of the laboratory fish anesthetizing system which incorporates a programmable database of voltage waveforms that are species and/or subject specific.

FIG. 7 is a flowchart of the laboratory fish anesthetizing system which incorporates a programmable database of voltage waveforms that are species and/or subject specific.

FIG. 8 is one physical configuration of the laboratory fish anesthetizing system.

LIST OF REFERENCE CHARACTERS

- 110—Tank
- 120', 120"—Electrodes
- 130', 130"—Wires
- 140—Programmable Voltage Source
- 160—Fish
- 180—Water Level
- 250—Impulse Voltage V_{max}
- 260—Impulse Voltage V_{min}
- 230—Impulse Voltage "t"
- 350—Periodic Voltage V_{max}
- 360—Periodic Voltage V_{min}
- 330—Periodic Voltage "t"
- 450—Dwell Voltage V_{max}
- 460—Dwell Voltage V_{min}
- 470—Dwell Voltage "t"
- 510—Proximity Detector
- 610—Voltage Mapper
- 620—Product to Waveform database
- 630—Waveform Input
- 720—Species Selection Step

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730—Subject Selection Step
 740—Load the Waveform from the Database Step
 750—Download the Waveform to the Voltage Source Step
 810—Laboratory Fish Anesthetizer
 820—Storage Unit
 830—Electrode Support
 840—Width Support
 845—Width Expansion
 850—Length Support
 855—Length Expansion
 860—Top Protector

DETAILED DESCRIPTION

Representative embodiments according to the inventive subject matter are shown in FIGS. 1-8, wherein similar features share common reference numerals.

The term “fish” refers to experimental fish used in a laboratory setting, which include, but are not limited to zebrafish. These fish typically belong to, but are not limited to, the taxa group Telostei or Teleostomi. The teleost fish include such fish as zebrafish (*Danio rerio*), medaka (*Oryzias*, sp.), fathead minnow (*Pimephales promelas*), or goldfish (*Carassius auratus*). It is well established that fish have a typical response to electrical fields applied in the water, although each individual fish and each type of species may have a varying response.

The term “tank” is generally known to those in the arts as a water tank, the preferred embodiment being a 10-40 gallon tank used by researchers that customarily sets on a laboratory top. Tank can also include larger tanks including outdoor tanks and naturally occurring ponds and streams. Also, the characteristics of the water should not be limited to freshwater, but, may also include water of differing salinities including sea water.

The term “electrical stimulation” refers to an electrical field impressed on the tissue of a fish in water. This electrical field will have a range in values that is dependent on the age and species of the fish.

The term “programmable voltage supply” shall mean a device that can output a range of voltages and currents in a waveform that is programmed either by hardware switch (e.g., a pulse generator) or by software (e.g. a computer controlled voltage generator).

Now referring to FIG. 1, a system 100 is shown for the anesthetizing fish. The system 100 has a water tank 110, two electrodes 120', 120" immersed in the water tank 110, two wires 130', 130" connected to the programmable voltage source 140. The water in the water tank 110 is filled to the water level 180. The electrodes 120', 120" are typically immersed below the water level 180. When voltage is applied across the two electrodes 120', 120" a voltage gradient is impressed across the fish 160. Depending on the size of the voltage gradient induced in the fish will determine the effect on the fish.

To anesthetize the fish 160, (electronarcosis), a voltage gradient of 150-250 V/m (1.5 to 2.5 V/cm) should be induced across the fish 160. To induce paralysis in the fish (electrotaxis) a greater voltage gradient than needed for electronarcosis should be induced across the body of the fish. To euthanize the fish 160, a voltage gradient of 1.5 to 5.0 V/cm or greater should be induced across the body of the fish 160. The voltage gradients needed for electronarcosis, electrotaxis, and euthanasia vary from fish species to fish species, and of course, differ based on the individual physiology of each fish.

In the laboratory setting, the water tank 110 will usually have dimensions of 2 feet in width, 4 feet in length, and 2 feet in height, holding the total water volume of 8 cubic feet or

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approximately 60 gallons. Freshwater conductivity in a laboratory tank ranges from 100 to 5000 S.

As previously mentioned, a fish typically used in laboratory biological studies is the zebrafish. Mature zebrafish grow to a size of approximately 6.4 cm. Juvenile zebrafish, which are more commonly used in research, range in size from approximately 0.9 to 1.5 cm.

The voltage potential varies as a matter of time and may be positive or negative on either electrode. The voltage potential may alternate in a manner such there is an equal balance of energy of time between the electrodes.

Now referring to an exemplary waveform as shown in FIG. 2, FIG. 2 shows an impulse waveform at the electrodes 120', 120" (as shown in FIG. 1) such that the impulse voltage Vmax 250 and the impulse voltage Vmin 260 are during the time period “t” 230. This impulse voltage Vmax 250 and impulse Vmin 260 should be of sufficient strength and duration to induce the physiological effects (e.g. electrotaxis and/or electronarcosis) on the laboratory fish.

Now referring to another exemplary waveform as shown in FIG. 3, FIG. 3 shows a periodic waveforms at the electrodes 120', 120" (as shown in FIG. 1) such that the periodic voltage Vmax 350 and the periodic voltage Vmin 360 are during the period time “t” 330. The periodic voltage Vmax 350 and the period voltage Vmin 360 should be of sufficient strength and duration to induce the preferred physiological effect (e.g. electrotaxis, electronarcosis, or euthanasia) on the laboratory fish.

Now referring to another exemplary waveform as shown in FIG. 4, FIG. 4 shows a periodic with dwell waveforms at the electrodes 120', 120" (as shown in FIG. 1) such that the dwell voltage Vmax 450 is separated by a dwell voltage Vdwell 470 and then followed by the periodic with dwell voltage Vmin 460 during the time period “t” 430. The periodic voltage with dwell should be of sufficient strength and duration to induce the physiological effects (e.g. electrotaxis and/or electronarcosis) on a laboratory fish.

Now referring to the system as shown in FIG. 5, FIG. 5 depicts a programmable voltage supply 140 connected to electrodes 120', 120" which are immersed in the tank 110. The programmable voltage supply 140 is connected to a proximity detector processor 520 and a proximity detector 510. The proximity detector 510 is used to determine if the fish 160 is too close to the electrodes 120', 120". If the fish is in close proximity to the electrodes, then the output waveform (see FIGS. 2,3,4) is modified to prevent injury to the fish 160 either by diminishing or eliminating the range of voltage from the programmable voltage supply 140.

Now referring to the fish anesthetizing system which is shown in FIG. 6, FIG. 6 depicts a programmable power supply 140 connected to electrodes 120', 120" which create an electric field in the tank 110 and across the fish 160. Connected to the programmable voltage supply 140 is a species to voltage mapping device 610. The species to voltage mapping device 610 stores a database of species electrical waveforms in a database 620. This database is updated by the input device 630.

The operation of the fish anesthetizing system of FIG. 6 is shown in the flowchart of FIG. 7. A species 720 or subject 730 waveform is selected and is loaded from a database 740. This waveform will have a voltage range and characteristics induces either electrotaxis or electronarcosis in the fish depending on the desired effect on the target species. Likewise the voltage range and characteristics depending on the subject profile, for example, if it is known that the target fish is a minnow that is 2 cm in length, this characteristic can be preselected from a database. Once the database voltage is

selected it is transferred (i.e. downloaded) **750** from the species to voltage mapper to the programmable voltage source.

Now referring to FIG. **8** that illustrates one configuration of the physical implementation of the laboratory fish anesthetizer **810**. The system has a storage unit **820** that contains the electronics and associated power supply. The storage unit is connected to the electrode supports **830** which are integrated with the length supports **850**. Interconnecting the two length supports are two width supports **840**. The length support **850** can be expanded lengthwise **855** to accommodate tanks of varying lengths. The width support **840** can be expanded widthwise **845** so that tanks of varying widths can be accommodated. Optionally present is a top protector **860** so that access to the top of the laboratory water is blocked.

The system in FIG. **8** may be configured to prevent access to the water in the tank while the apparatus is operating. Those skilled in the arts will recognize that interlocking switches may be employed so that the electrodes **120'**, **120"** of the laboratory fish anesthetizer **810** are not energized if power is applied.

Persons skilled in the art will recognize that there are a wide variety of databases that can be used to store signal patterns, including, but not limited to SQL databases, text databases, or object oriented databases. Persons skilled in the art will recognize that there are a wide variety of programmable voltage supplies, including, but not limited to products manufactured by Lamda (Neptune, N.J., USA). It is also understood by those skilled in the art that the database and/or the programmable power supply may be implemented in firmware in a manner to reduce and/or minimize costs.

Persons skilled in the art will recognize that many modifications and variations are possible in the details, materials, and arrangements of the parts and actions which have been described and illustrated in order to explain the nature of this inventive concept and that such modifications and variations do not depart from the spirit and scope of the teachings and claims contained therein.

All patent and non-patent literature cited herein is hereby incorporated by references in its entirety for all purposes.

The invention claimed is:

1. A fish anesthetizing apparatus that induces a potential field across the body of a laboratory fish comprising:
 - a programmable power supply, the programmable power supply having a first output terminal and a second output terminal; said first output terminal and said second output terminal capable of creating a potential field;
 - a pair of electrodes, the first electrode connected to the first output terminal and the second electrode connected to the second output terminal;
 - a species to voltage mapping device, the species to voltage mapping device further comprising a database of a plurality of prestored species electrical waveforms; wherein selection of a prestored species electrical waveform from the database of a plurality of prestored species electrical waveforms is operably downloaded into the programmable power supply;
 - a laboratory water tank, the laboratory water tank capable of receiving the pair of electrodes and a multiplicity of fish, each fish having a body; wherein when the programmable power supply is operated, the potential field is impressed across the body of each fish; so that a physiological state is induced in the multiplicity of fish selected from the group consisting of the physiological states of deterrence, electrotaxis, electronarcosis, and electroeuthanasia.
2. The apparatus of claim 1 where the species electrical waveforms has a periodic waveform with a frequency from 0.1 to 1000 Hz.
3. The apparatus of claim 2 where the species electrical waveforms ranges from 1.5V/cm to 2.5 V/cm across the body of each fish.
4. The system of claim 2 where in the maximum potential difference greater than 5.0 V across the body of each fish.
5. The apparatus of claim 1 where the species electrical waveforms is an impulse.

* * * * *